E. F. Butler

TECHNICAL BULLETIN No. 9. FEBRUARY, 1909

From the author

New York Agricultural Experiment Station.

GENEVA, N. Y.

IMPERIAL BUREAU OF MYCOLOGY
28 JUN. 1921

# A MYCOSPHÆRELLA WILT OF MELONS.

J. G. GROSSENBACHER.

PUBLISHED BY THE STATION.

## BOARD OF CONTROL.

GOVERNOR CHARLES E. HUGHES, Albany.
COMMISSIONER RAYMOND A. PEARSON, Albany.
STEPHEN H. HAMMOND, Geneva.
LYMAN P. HAVILAND, Camden.
EDGAR G. DUSENBURY, Portville.
THOMAS B. WILSON, Halls Corners.
IRVING ROUSE, Rochester.
ALFRED G. LEWIS, Geneva.

## OFFICERS OF THE BOARD.

THOMAS B. WILSON, *President.*
WILLIAM O'HANLON, *Secretary and Treasurer.*

### EXECUTIVE COMMITTEE.

STEPHEN H. HAMMOND, LYMAN P. HAVILAND,
THOMAS B. WILSON.

## STATION STAFF.

WHITMAN H. JORDAN, SC.D.,LL.D., *Director.*

GEORGE W. CHURCHILL, *Agriculturist and Superintendent of Labor.*
WILLIAM P. WHEELER, *First Assistant (Animal Industry).*
FRED C. STEWART, M.S., *Botanist.*
JOHN G. GROSSENBACHER, PD.B., A.B., *Associate Botanist.*
G. TALBOT FRENCH, B.S., *Assistant Botanist.*
LUCIUS L. VAN SLYKE, PH.D., *Chemist.*
ALFRED W. BOSWORTH, B.S.,
ERNEST L. BAKER, B.S., *Associate Chemists.*
ARTHUR W. CLARK, B.S.,
ANTON R. ROSE, B.S.,
MORGAN P. SWEENEY, A.M.,
JAMES T. CUSICK, B.S.,
OTTO MCCREARY, B.S., *Assistant Chemists.*
HARRY A. HARDING, M.S., *Bacteriologist.*
MARTIN J. PRUCHA, M.S., *Associate Bacteriologist.*
JAMES K. WILSON, B.S., *Assistant Bacteriologist.*
GEORGE A. SMITH, *Dairy Expert.*
FRANK H. HALL, B.S., *Editor and Librarian.*
PERCIVAL J. PARROTT, M.A., *Entomologist.*
HAROLD E. HODGKISS, B.S.,
WILLIAM J. SCHOENE, B.AGR., *Assistant Entomologists.*
ULYSSES P. HEDRICK, M.S., *Horticulturist.*
RICHARD WELLINGTON, B.S.,
MAXWELL J. DORSEY, B.S.,
W. H. ALDERMAN, B.S.AGR., *Assistant Horticulturists.*
ORRIN M. TAYLOR, *Foreman in Horticulture.*
*F. ATWOOD SIRRINE, M.S., *Special Agent.*
†JENNIE TERWILLIGER, *Director's Secretary.*
FRANK E. NEWTON,
WILLARD F. PATCHIN,
CORA A. WHITAKER, *Clerks and Stenographers.*
ADIN H. HORTON, *Computer and Mailing Clerk.*
JULIA H. HOEY, *Junior Clerk.*

Address all correspondence, not to individual members of the staff, but to the NEW YORK AGRICULTURAL EXPERIMENT STATION, GENEVA, N. Y.

The Bulletins published by the Station will be sent free to any farmer applying for them.

---

*Riverhead, N. Y.
†Absent on leave.

## CONTENTS


| | Page. |
|---|---|
| Introduction | 196 |
| Plan of the bulletin | 196 |
| Résumé of Chester's articles on "Anthracnose of watermelon" | 197 |
| Résumé of Smith's article on "Leaf-spot on Cucurbits" | 199 |
| Comparison of Chester's and Smith's work | 201 |
| Observations on herbarium material from Del. Exp. Sta | 204 |
| The writer's work of 1907 | 205 |
| The disease noticed | 205 |
| General description of the disease | 207 |
| Making pure cultures from diseased melon vines | 207 |
| Preliminary inoculations in the greenhouse | 208 |
| Inoculation of melon vines in the garden | 209 |
| Microscopic characters of the pycnidial form | 209 |
| Discharge of pycnidiospores and how the disease was spread | 210 |
| Another spore form found | 211 |
| Van Tieghem cell cultures of pycnidiospores | 212 |
| Cell cultures of ascospores | 212 |
| Ascosporic and pycnidiosporic inoculations of muskelon | 213 |
| Inoculation of pumpkin vines with both mycelia | 214 |
| Some peculiarities of pumpkin as a host | 215 |
| Inoculation of watermelon vines | 215 |
| Comparision of susceptibility of muskmelon and watermelon | 215 |
| Inoculation of cucumber vines | 216 |
| Why is the cucumber immune? | 216 |
| Inoculation of young muskmelon plants with spores | 217 |
| Spores, in culture, not typical | 218 |
| Re-examinaion of the Mycosphærella wilt of melons, in 1908 | 219 |
| The appearance of other parasitic fungi | 219 |
| The reappearance of the red spider | 219 |
| The reappearance of the Mycosphærella wilt | 219 |
| No Phyllosticta found on the leaves | 220 |
| Re-isolation of the fungus in 1908 | 220 |
| Mycelial inoculation of other species of cucurbits (1908) | 221 |
| Development of perithecia on naturally immune cucurbits | 222 |
| Ascosporic and pycnidiosporic inoculation of muskmelon | 224 |
| A probable reason the disease is not found in fields of western N. Y. | 225 |
| Remarks on the work of 1907–8; preventive measures | 225 |
| Conclusions regarding the names of the fungus | 225 |
| Technical descriptions of the fungus | 226 |
| Appendix | 227 |

TECHNICAL BULLETIN No. 9.

# A MYCOSPHÆRELLA WILT OF MELONS.[1]

J. G. GROSSENBACHER.

## SUMMARY.

The muskmelons, in one of the Experiment Station greenhouses, were much damaged during 1907-8 by an uncommon disease. The vines were parasitized by a fungus (Mycosphærella) shortly before the earliest melons were ripe. The disease was preceded, in both instances, by the attacks of a red spider, though in 1908 the damage by the spiders was but slight. The fungus is reported as causing a disease of several cucurbits in the fields of some parts of Delaware, but had never been recorded for this State. Since the Delaware reports differed in some respects from the observations made at this Station in 1907, nothing was published till further information could be obtained on the points of difference. The observations were repeated and extended in 1908.

The pathogeneity of the fungus and the relation of its two spore-forms are established. Inoculation experiments were effective, and, with but a few interesting exceptions, the spore-forms of the fungus always appeared successively on the inoculated vines.

The disease will probably not become injurious to field melons in western New York, though in greenhouses it may cause much trouble.

[1] The more or less meaningless names in plant disease literature, as designations of both parasitic and non-parasitic diseases, have been the source of much annoyance to the writer, since the cause of a disease is not suggested by its name. The plea for such terms as wilt, blight, leaf-spot, canker and rot, is that the laymen can more readily understand them, but since only a very small fraction of plant disease literature is read by them, it seems unfair to the pathologist to compel him to use such vague terminology on their account. This need seems to be felt by a number of plant pathologists, as evidenced by some generic-name and disease-character combinations in some experiment station bulletins and other places, but not enough prominence has been given to the matter

The suggestion is therefore made that the genus name of the infesting parasite be combined with some popular, descriptive term, as in this bulletin, or with the word *disease*, e.g. Sphæropsis canker of apple, Bacillus canker of apple, Tilletia smut of wheat, Ustilago smut of wheat, Fusarium wilt of flax, Bacillus wilt of cucurbits, Botrytis rot of lettuce, Sclerotinia rot of lettuce, and Thielavia root-rot of tobacco. Of course there are some popular names now in use, which are fairly distinctive and useful. The names of diseases caused by malnutrition and other physiological disorders may not so readily be made more concise until their causes are ascertained.

## INTRODUCTION.

A striking disease of muskmelon (*Cucumis Melo*) made its appearance in one of the Station greenhouses devoted to melon culture during the summer of 1907. It was first noted by Mr. R. Wellington, Assistant Horticulturist, who was carrying on experiments in variety testing of muskmelons. Both the imperfect and ascosporic forms of fungous fructifications were found on the diseased vines, and pure cultures of them were used for inoculating healthy plants. The fungus proved to be the cause of the disease, and the associated fruits were shown to belong to one and the same fungus. This work was finished and written up before some published accounts by Mr. F. D. Chester[2] and Mr. C. O. Smith,[3] seemingly on the same disease, were found. The publication of the bulletin was stopped, for the time being, because it did not agree in all particulars with the articles cited; re-examination seemed necessary. In midsummer, 1908, the disease reappeared in the same greenhouse, thus affording an opportunity to test the former work.

*Plan of this bulletin.*—After an examination of Chester's and Smith's articles relating to this disease, with frequent quotations from the parts most essential, they are briefly compared with each other. The writer's observations and experiments of 1907–8 are then recorded, followed by the comparison of results. An attempt is made to correct the nomenclature of the fungus causing Mycosphærella wilt of melons. Finally, to clear up Mr. Smith's last paragraph, an appendix is added on the relation of the names of some cucurbit fungi.

---

[2] Del. Exp. Sta. 5th Ann. Rpt. pp. 75–9 (1893), Newark, Del.; also *Bul. Torr. Bot. Club* **18**: 373–4 (1891), N. Y. Botanic Garden.

[3] Del. Exp. Sta. Bul. 70, (1905), Newark, Del.

## RESUME OF CHESTER'S WORK[4] ON AN "ANTHRACNOSE OF THE WATERMELON."

During the autumn of 1890, a diseased watermelon vine was sent Mr. F. D. Chester at the Delaware Experiment Station from some point in that state, and he describes it as follows: "Where the vine stalk was yet green, it was found to be covered with elongated dark patches and irregular areas; tendrils, buds and leaf stalks were invariably black and dead, the leaves were covered with black blotches, marked in many cases, by more or less distinct eccentric rides."[5] He recognized "pustules" on the leaf-spots as fruits of a Phyllosticta.

The vine was thrown upon a rubbish heap out of doors, where it was seemingly left until 1892 and then used again for inoculation purposes; though from another place[6] it seems that the material, used for the inoculation in 1892, must have been collected in 1891, because the disease is said to have been uncommonly destructive during 1891.[7] To quote: "The disease attacks leaf, leaf stalk, stem, tendrils, fruit buds and blossoms." The spots on the stems, which later become white in the center, are said not to cause any very serious damage.

It is further stated that "the fungus causing the above trouble is a member of the genus Phyllosticta, although from the character of the sporules, which are sometimes uniseptate and hyaline, it is questionable whether it might not, following Saccardo, be classed as an Ascochyta."[9] The following description and name are given: "*Phyllosticta citrullina*, n. sp. Spots circular, irregular, black, concentrically ridged, becoming confluent. Pycnidia amphigenous, brown, immersed, scarcely erumpent, membranaceous, lenticular 75–131 $\mu$, average of many measurements 107 $\mu$ x 67 $\mu$. Sporules 9–10.7 $\mu$, average about 10 $\mu$ x 3.5 $\mu$, generally continuous, sometimes uniseptate,

[4] Del. Exp. Sta. 5th Ann. Rpt. pp. 75–9, (1893).
[5] l. c., p. 75.
[6] Blight of watermelon vines—*Bul. Torr. Bot. Club* **18**: 373–4, (1891).
[7] l. c., p. 373.
[9] l. c., p. 374.

straight, slightly curved, ends obtuse, often biguttulate, hyaline. On leaves and other parts of watermelon."[10]

As stated before, the watermelon vine sent Mr. Chester in the autumn of 1890 is presumably used to inoculate young watermelon plants in 1892, according to the article in the annual report previously cited, but it seems more plausible to assume that the material used was collected in 1891, as suggested by the prevalence of the disease during that season. At any rate, during the early part of 1892 young watermelon plants were inoculated with spores obtained from an old diseased vine. Spots appeared on the leaves and later Phyllosticta pycnidia.

*A Mycosphærella found.*—Watermelon leaves, which had been preserved in press, were put into a moist chamber. In 10–14 days pycnidia of the same Phyllosticta appeared on the leaf-spots, and among them, perithecia of a Mycosphærella with spores measuring 14 x 5 μ.

Mr. Chester studied the disease in the field during 1892. On some fresh, characteristically diseased plants, he found also a "pink anthracnose on the same spots." The anthracnose is presumed to be *Glœosporium lagenarium* (Pass.) Sacc. et Roum.,[11] and is said "to agree with No. 2448 b. in Ellis' N. A. Fungi, which is there marked *C*[*olletotrichum*] *lagenarium* Pass. var. *follicolum* E. & E."

"To prove the relationship of the Colletotrichum to the disease under discussion, several young watermelon plants growing in the field were on July 28 thoroughly atomized with water containing in suspension Colletotrichum spores taken from watermelon rind. On August 3 the plants began to show the characteristic appearance of the disease and in less than one week were entirely dead. The check unatomized plants remained perfectly healthy."[12] Similar inoculations were re-

[10] l. c., p. 374.

[11] *Collectotrichum lagenarium* (Pass.) Sacc. et Roum. is said to be identical with *C. oligochætum* Cavara, given out as No. 99 in Briosi et Cavara's Funghi Parassiti, and not No. 100, as stated by Mr. Chester in a foot note on p. 78, Del. Exp. Sta. 5th Ann. Rpt. and by Dr. Halsted on p. 350, N. J. Exp. Stas. Rpt. for 1893, New Brunswick, N. J.

[12] Del. Exp. Sta. 5th Ann. Rpt. p. 78.

peated on large watermelon plants in the field, using "spores taken from the pinkish crust on watermelon rind. In two weeks many leaves showed characteristic spots, the vine stalk showed discolored zones here and there."

In order to obtain perithecia "numerous specimens of diseased rind" were collected and put into a wintering cage, but the Colletotrichum remained unchanged, and no perithecia appeared.

## RESUME OF SMITH'S WORK[13] ON A "LEAF-SPOT ON CUCURBITS."

Mr. Smith notes the abundance of a "leaf-spot" of cucurbits on the leaves and fruits of squash and pumpkin, and "to a limited extent on the leaves of cucumber and cantaloupe." He thinks it identical with the watermelon leaf-spot disease caused by *Phyllosticta citrullina* Chester.[14] "On the leaves of various cucurbits, there appeared a leaf-spot which was quite similar on the different species. This appeared as dead spots from 0.5–1 inch (1–2 cm.) in diameter. . . . It is thought these spots may be associated with the melon anthracnose, *Colletotrichum lagenarium*, but no conclusion can be drawn that the two are connected in any way. The disease is also found on the vine. Plate I, E."[15] "The fungus on the stem forms a whitish area which is darkened with blackish specks, the pycnidia, Plate I, E."[16] "The spores are hyaline, oblong or oblong-elliptical, uniseptate bodies . . . vary somewhat in size and are not always septate and probably never all become so."[17] In pure cultures they were found to be mostly continu-

[13] Del. Exp. Sta. Bul. 70 (1905), Newark, Del.

[14] F. D. Chester, *Bul. Torr. Bot. Club* **18:** 373–4 (1891), also Del. Exp. Sta., 5th Ann. Rpt. pp. 75–9.

[15] Del. Exp. Sta. Bul. 70, p. 4.

[16] Reproduced in this Bul. as fig. E of Plate III. The negative was borrowed from Del. Exp. Sta., through the kindness of Dr. Mel. T. Cook, Mycologist at that station.

[17] l. c., p. 5.

ous in their early stages, and biguttulate to sparsely septate, later.

Squash agar, potato agar, squash plugs and stems were used as culture media. "When growing on plugs or stems of squash, the mycelium is whitish and floccose; but with age changes to a dark color. Experience has shown that, on the rind, the pycnidia are formed most abundantly."[18]

"Seedlings of several varieties of squash, pumpkin, cantaloupe, watermelon and cucumber were inoculated with fresh spores during the winter and spring months. This was done not only with the fungus found growing on the individual host, but also cross inoculations were made on the other hosts of the cucurbits. The results were most satisfactory and conclusive *i. e.* that all these several fungi were really one and would grow on any of the cucurbits used in the experimental work."[19] "The *Ascochyta* form of the fungus, grown from the ascosporic stage, was found to cause the disease on cucurbits like the other *Ascochyta* grown from *Ascochyta* spores."[20] "Also a species of *Ascochyta*, resembling the one studied, grow[n] from an ascosporic or perfect stage, a species of *Sphacrella*, gave equally positive results in inoculation work."[21]

Mr. Smith expresses the view that since an Ascochyta differs from a Phyllosticta or Phoma only in its having septate spores, it may easily be mistaken for one of the continuous spored genera. He thinks it probable that *Phyllosticta orbicularis* E. & E.[22] "may be the same fungus."

*A Mycosphærella associated with pycnidia.*—A Mycosphærella was sometimes found associated with the Ascochyta *on squash stems.* "Attempts were made to germinate the ascospores . . . but only on one occasion were they seen to germinate. . . . Agar plates were poured, and these were inoculated at several points with a single perithecicum of the

[18] l. c., p. 5.
[19] l. c., p. 6.
[20] l. c., p. 10.
[21] l. c., p. 7.
[22] The N. Am. Phyllostictas(1900).

*Sphaerella* stage. . . . This experiment was repeated perhaps a dozen times always with the same result. The perithecia of the *Sphaerella* stage are darker and more carbonaceous than the pycnidia of the Ascochyta stage, so there was no danger in confusing the two. The fruit produced by this inoculation was always examined and on one occasion the *Sphaerella* and *Ascochyta* stage were found in the same colony on the agar plate. In fact, at least, two of the *Sphaerella* perithecia were found."[23]

Smith's article on the "Leaf spot on Cucurbits" is closed with the following paragraph in small type. "*Sphaerella cucurbitaceorum* (Schw.). This fungus has been described in Saccardo's Sylloge under the genus *Laestadia*. At the close of his description he suggests in a doubtful manner that the spores are at length uniseptate. This I believe to be true and hence the species is a Sphaerella. This fungus seems to be much like one described by Fries as Sphaeria cucurbitacearum, but from his meagre description the identity is uncertain. I don't think there is the least question, but that the perfect stage, or squash *Sphaerella*, is identical with *Laestadia* cucurbitacearum (Schw.) and should be, named Sphaerella cucurbitacearum (Schw.) although in my text I have been satisfied to call the fungus *Sphaerella citrullina*."[24]

## COMPARISON OF CHESTER'S AND SMITH'S WORK.

Both Chester and Smith seem to give undue prominence to the leaf spot Phyllosticta, though the fungus on the stem is noted by Chester as forming "elongated dark patches" and Smith says it "forms a whitish area which is darkened with blackish specks, the pycnidia." The Mycosphaerella-wilt fungus with its characteristic appearance and numerous fruiting bodies on the stem is given but secondary place by Smith and scarcely noticed at all by Chester. However, the latter's view seems to have changed during the progress of his work as shown by his change of names, first calling it, "Wilt of watermelon vines," and then "Anthracnose of the watermelon."

[23] l. c., p. 9.
[24] l. c., p. 10.

Chester tried to show that his Phyllosticta is the same as a certain Colletotrichum; he succeeds in showing that the "pink anthracnose" is parasitic on cucurbits, but fails to connect it with his Phyllosticta. Smith tried to connect his Ascochyta with Chester's *Phyllosticta citrullina* but records only conjectural evidence.[25] The remarks by Chester on the relation of several anthracnoses, and his Phyllosticta, and the work and suggestions by Halsted,[26] on the former, need not be considered here, because no relation could be found between them and the Mycosphærella-wilt fungus.

The production of dead areas on leaves of a host, by pouring water laden with spores of a stem parasite of the same host on them, is no proof that the fungus normally parasitizes the leaves.[26] Especially is this true when the dead areas or spots produced fail to develop the fruits of the fungus used. In cases where the inoculated plants are enclosed in moist chambers, the results are yet more unreliable. When aerial parts of a host are at all susceptible to a certain fungous parasite, it seems probable that, under such conditions, all aerial tissues of that host, which have no special structural protection, would be more or less attacked, unless the required aeration for the fungus were prevented by the excessive moisture. Smith records no fruits on his artificially induced leaf spots; nor has the writer obtained pycnidia on leaves under any conditions, though dead areas developed from moist chamber infections; see Plate V.

It seems that Chester either overlooked the fructifications of the Mycosphærella wilt on the vines, or it was not associated with his Phyllosticta leaf-spot, because he makes no mention of pycnidia or perithecia on vines; though, according to the writer's observations, to be given later, that is a constant character of the disease, both in the greenhouse and in the field. It appears more probable that Chester found the two fungi on the

[25] N. J. Exp. Stas. Rpt. (1893) pp. 347-56, New Brunswick, N. J.

[26] Miss C. M. Gibson's infection experiments with various rusts upholds such a view. Notes on infection experiments with various Uredineæ. *The New Phytologist* **3**: 184–191 (1904).

same plants in Delaware, and that, since both are parasitic on the same host, they were confused with each other. Chester's characterization of the disease, and his finding a Mycosphaerella seem to indicate that he also had Smith's fungus. From one of Smith's sentences, quoted on page 200, it seems possible that he had two fungi of the same type; on that assumption Chester may have had the other Mycosphaerella. However, that suggestion is probably incorrect, though the quoted sentence is too ambiguous to be certain of its meaning.

The relation of Smith's ascosporic and pycnidial forms of the parasite is definitely shown, and sufficient scattered description of both is given, so that, with the aid of his photograph of a diseased squash stem (Plate III, fig. E, of this bulletin) and a large quantity of squash stem material, obtained from the Delaware Station in 1907, (collected and labeled by C. O. Smith) there is no doubt that Smith had the same fungus which is discussed in this publication, though his technical description of the imperfect form should be discarded, because it is only an amended form of Chester's description of *Phyllosticta citrullina*. As indicated before, *Phyllosticta citrullina* Chester seems only an accidental associate of the Mycosphaerella-wilt fungus, and has never been found by the writer.

According to the evidence obtained, there is no reason to suppose that the Mycosphaerella-wilt fungus is at all related to *Sphaerella (Lasfadia) Cucurbitacearum* (Schw.) ? Cooke (see appendix), but it seems possible that *Sphæria Cucurbitacearum* Fries may be a Phoma[27] often found on the rind of squashes, etc. At any rate, spore measurements attached to that species by Saccardo,[28] and seemingly copied by Allescher,[29] correspond very nearly to this Phoma, the spores of which measure 7–10 $\mu$. Whether or not this rind Phoma is a form of Cooke's *Sphærella (Lasfadia) Cucurbitacearum* can not be determined with-

[27] Material of this type was obtained from the Mycologists at the Delaware Experiment Station, in 1907, which was collected and labeled Ascochyta, by C. O. Smith, Dec. 1903, at Newark.

[28] Sylloge Fungorum, III, p. 148.

[29] Rbh. Kr.—Flora, Vol. I, pt. 6, p. 284.

out examining all authentic herbarium material (if there is any) in connection with extended field observation and cultivation of the above Phoma.

## OBSERVATIONS ON HERBARIUM MATERIAL[30] RECEIVED FROM THE DELAWARE EXPERIMENT STATION, 1907.

Squash vine material labeled Sphaerella had rather scattered perithecia, but of the same type and structure as those of the Mycosphaerella wilt fungus. The measurements were: perithecia 110-170 $\mu$ asci 45-55 × 7-10 $\mu$, and spores 10-13 × 4 $\mu$. Squash *stem* material *marked* "*Ascochyta citrullina*" was found to *contain mostly perithecia* of the Mycosphaerella wilt fungus, but also some of the *typical Diplodina pycnidia*, with spores hyaline, 9-15 $\mu$ long, uniseptate, more or less cylindrical and with or without constrictions at the septa. The Mycosphaerella perithecium, asci and spore characters were the same as those of the Mycosphærella-wilt fungus.

The cucurbit *leaf* specimens labeled "*Ascochyta citrullina* (Ches.) Smith" had pycnidia measuring 80-135 $\mu$; the spores were elongate-ovoid, hyaline, continuous (about 3 per ct. being doubtfully septate) and measuring 7-9 × 3 $\mu$. It seems a fairly typical Phyllosticta.

There were also a few good specimens marked "Colletotrichum," on leaf petioles of squash. The fruiting regions on these petioles were typical of that genus. The setæ were very conspicuous and the spores of the required type.

A considerable quantity of material marked "Ascochyta on fruit of squash, collected by C. O. Smith, December 1903, Newark, Delaware," may be described as follows: Pycnidia irregularly scattered on discolored rind or in concentric rings on nearly unchanged and normally colored rind. Both types are covered by the unbent palisade-like layer of the squash-fruit epidermis; only the blackish ostiola bend and break

[30] Collected mostly by C. O. Smith, and lent by the Mycologists of the Delaware Exp. Sta.

through it. They measure 90–150 $\mu$ and are more or less depressed by the epidermis. The spores are oblong-ovoid, hyaline and mostly continuous, measuring 7–10 $\mu$, the longest ones often being uniseptate. Probably a Phoma as indicated before.

These, together with the greenhouse and field observations, to be given later, seem to show that Chester's *Phyllosticta citrullina* may be retained in the genus Phyllosticta, and that it at least should not be connected with Smith's "*Ascochyta on squash stems.*" However, nothing can be said about the real relation of *Phyllosticta citrullina* Ches. and Smith's "Ascochyta" on squash rind, except that according to the accepted scheme of classifying Deuteromycetae they belong to different form genera, that on the rind being morphologically a Phoma.

The material marked "Ascochyta on squash stems" probably underwent some change *after* it was *examined* and *labeled*, since it had mostly perithecia of the Mycosphaerella wilt fungus. Some of the writer's observations also indicate that the pycnidia of this fungus may later change to perithecia by the development of asci from the interior of the basal portion of the fruiting body. In fact the mixture of these perithecia and pycnidia of the Mycosphaerella-wilt type, under the label Ascochyta by its own author, indicates, not only that Smith's Ascochyta and Sphaerella are connected, but also, that he had the fungus under discussion in this bulletin.

## THE WRITER'S WORK OF 1907.

As intimated in the introduction of this bulletin, the work, on this disease producing fungus, had been completed and the discussion put in type before any reference to it had been found elsewhere. The following is simply a scissors-and-paste report of the work of 1907; the present year's (1908) re-examination of the disease and of the fungus causing it, is briefly given afterwards.

### DISEASE NOTICED.

The muskmelon vines in the Station greenhouse had practically reached their full growth before any trouble appeared.

Some of the first fruits were nearly ripe. The fungous disease was preceded by a rather severe attack of the red spider (*Tetranychus telarius*) upon the foliage, to such an extent, in fact, as to kill many leaves. The spider was similarly troublesome in an adjoining greenhouse in which cucumbers (*Cucumis sativus*) were grown. The blotched and dying leaves of cucumbers and melons first brought to the pathology laboratory by Mr. Wellington showed no traces of any fungous parasite. Yet the leaves continued turning yellow and dying in both greenhouses. The leaf petioles of these leaves slowly turned yellow and dried up, beginning at the dead leaf blade and gradually proceeding toward the vine axis from which the leaf grew. The spider was combatted by using ordinary hydrant water applied to the infested parts of the plants with a hose. It withstood that treatment but comparatively few days.

But it seems the trouble had only just begun. After the ravages of the spider had been gotten somewhat under control there appeared a marked difference between the recovering abilities of the plants in the cucumber and melon houses. The former soon showed fresh green foliage again, and, with the exception of the dead leaves, looked normal; while the latter seemed not to recover very rapidly. In fact, in a few cases, the dying petioles seemed to have a somewhat *water logged* appearance at their junction with the vine. On a few nodes of this kind an *oily-green colored region* was evident from which a few drops of *resin colored gum* had exuded. However as other things required attention at this time no more thought or time was given the melons till about a week later, when a few internodes, which had the nodal regions rather thickly specked with light-brown to dark-brown fruiting bodies of some fungus, were brought to the laboratory. On the internodes beyond the fruiting zones, the oily-green, water-logged character of the vine was very conspicuous; and this, with the resin-colored drops of gum which had exuded from them, seemed evidence that the condition of some nodes, as noted before, was the beginning of this disease.

Photo. by M. J. Dorsey

PLATE I.—A DISEASED BUT STILL LIVING MUSKMELON VINE.

1, leaves killed by red spiders; 2, diseased regions with dead cortical girdles having pycnidia, bordered by dying, oily green colored tissues.

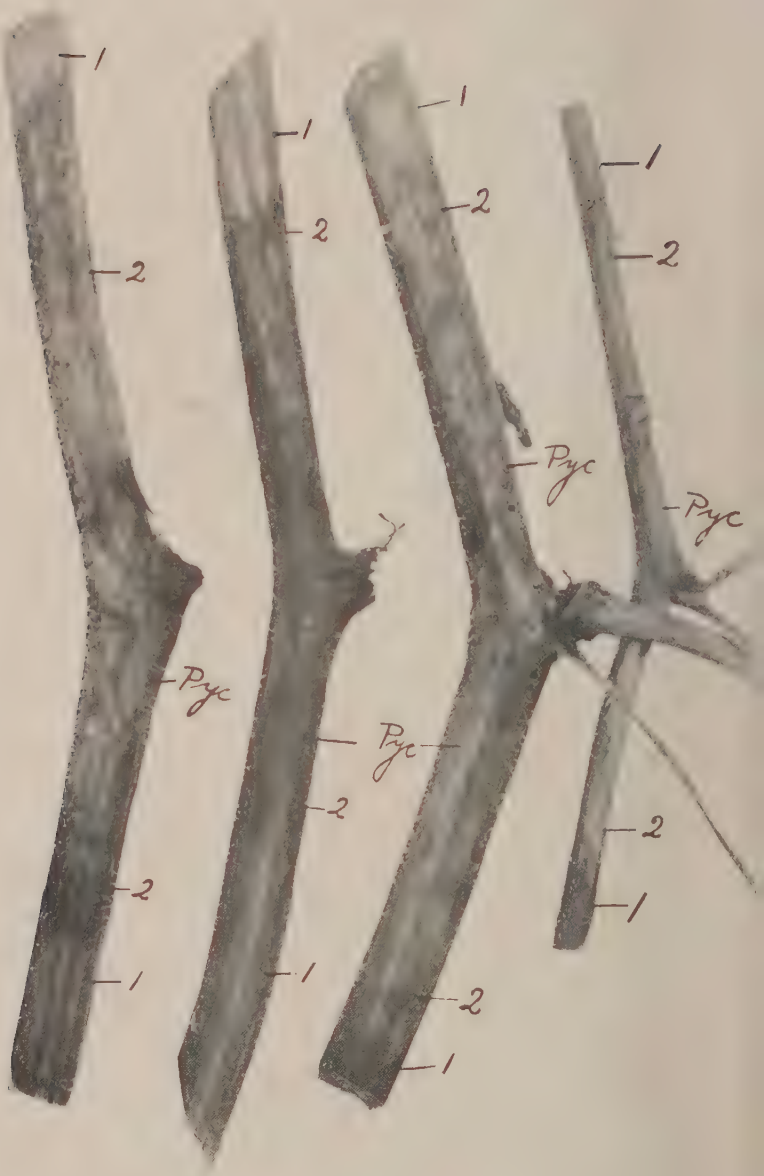



Photo. by M. J. Dorsey

PLATE II. - PIECES OF VINE SHOWN IN PLATE I, WITH NUMEROUS PYCNIDIA (PYC
1, Green, living portions; 2, regions with dead cortical tissues.

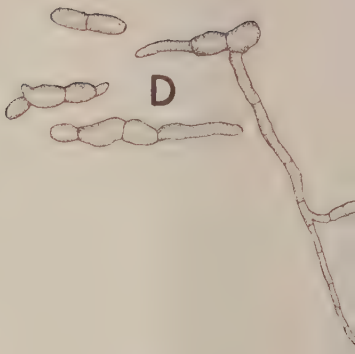
PLATE III.

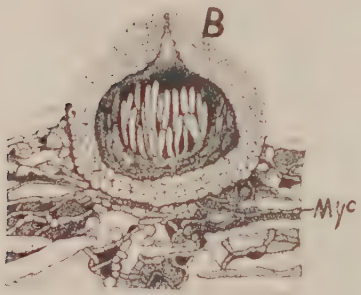
A, Portion of muskmelon vine with both pycnidia and perithecia: 1, Green, living portions; 2, dead, cortical regions, with pycnidia; 3, regions with perithecia.

Photo. by M. J. Dorsey.

E, diseased squash stems, showing black dots, the pycnidia of the fungus."

From Del. Ex. Sta. Bul. 70, Plate 1.



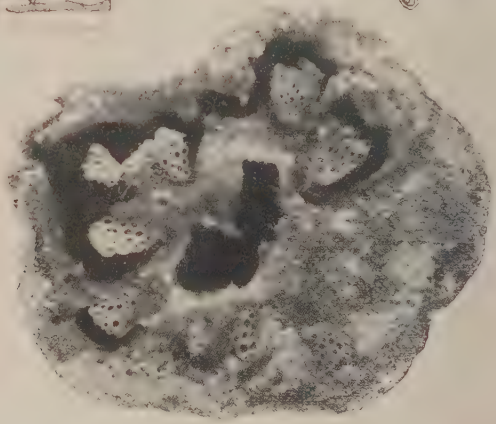


PLATE IV.

A.—Section of pycnidium on muskmelon, showing mycelium (myc.), epidermis host (E) and spores.

B.—Section of a perithecium with immature asci. On muskmelon (cut slightly the bias, walls are really a little thinner).

C.—An ascus with spores, and the three types of spores.

D.—Germinated and ungerminated pycnidiospores.

E. Photograph of section of muskmelon vine, showing isolation of fibrovasc bundles, and some fungous-hyphal masses.

ATE V -YOUNG MUSKMELON PLANT INOCULATED, IN MOIST CHAMBER, WITH PYCNIDIOSPORES BY POURING THEM ON THE LEAVES AND STEM.

X, Affected regions; no fruiting bodies developed on the leaves.

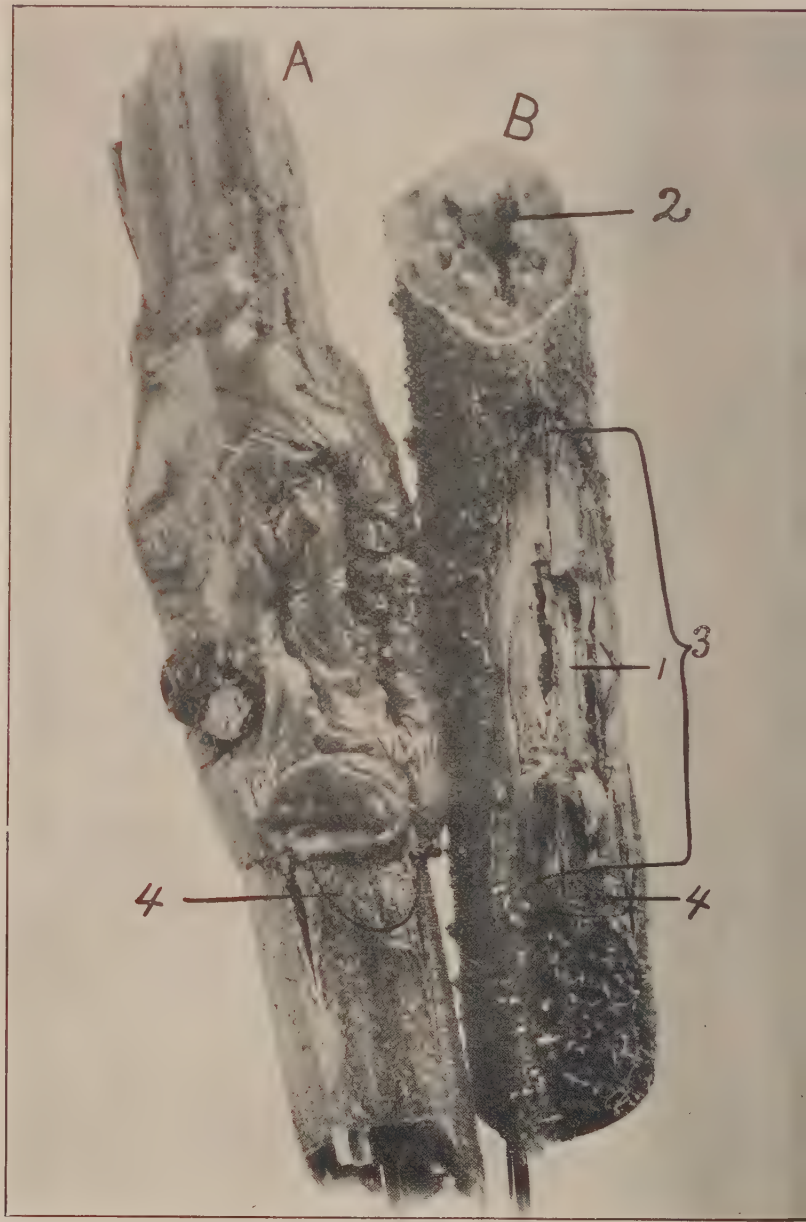



Photo. by F. E. Colburn

PLATE VI.—SHORT PIECES OF INOCULATED PUMPKIN VINES.

A, Grafting wax still in place.

B, wax removed from region 3: 1, wound through which mycelium entered hollow pith shown at 2; 4, dead portions of partially detached slivers, with perithecia.

INVESTIGATION OF DISEASE.

*General description of the disease.* The greenhouse was visited at once and a striking difference found between the appearance of the melon plants then, and that of a week previous. Three or more plants were much wilted. Several of their lower nodes and some of their internodes were oily green in color with or without gum exudation, while others had passed beyond that stage, having all the formerly oily-green regions turned either a dark and gummy or dry and grey color, depending upon the relative quantity of gum present and the extent of the cortical and subepidermal mycelial development that had taken place during the earlier stages of the disease. Upon looking the plants over more carefully it became very evident that a large percentage of them had some stage of the trouble at one or more of their nodal regions, more commonly on the lower nodes. Most of these infections were confined to small regions at the nodes, having seemingly originated in the axil of the leaf or in the crotch of a branch. All the smaller infections simply had the peculiar water-logged and oily-green condition; however, at those places where the disease had extended some distance above and below the nodes, there were generally numerous drops of gum and pycnidia present, especially on the lower nodal regions of the vine. See plates I and II. In no case was an infection found to have started on an internode, but always either in the axil of a leaf or crotch of a branch. All infections observed at branchless nodes occurred at those having leaves which had been affected more or less by the red spider. At branching nodes the infection took place nearly exclusively in the crotch and not from the leaf axil; in some cases of crotch infection the leaf was normal and healthy.

*Making pure cultures from diseased melon vines.*—An extensive set of pure cultures was made from various regions of diseased muskmelon vines by taking bits of tissue under sterile conditions and transferring them to potato-agar tubes. All tubes containing tissue from the pith region remained sterile,

while those from the cortical region developed fungous mycelium in every case.

*Preliminary inoculations in the greenhouse.* There being still some unaffected plants in the greenhouse, a few of them were used for preliminary inoculations. Two of the healthy vines were inoculated at two points each, by cutting a bit of the cortical tissue loose and inserting bits of mycelium from the potato-agar tubes, taking the usual precautions regarding sterile instruments. After sterilizing the scalpel carefully, two additional wounds were made. All were covered with grafting wax. Three days later both the inoculated vines were slightly wilted, and a week after the date of inoculation the infested vines had the characteristic appearance of the disease both above and below all four of the inoculated points; the usual oily-green, water-logged appearance of the affected regions farthest removed from the points of infection, and drops of gum on the nearer, darker colored parts. Next the wax, all the vines had an abundance of the yellowish brown to dark brown fruiting bodies. The checks, on a vine next the inoculated ones, had no trace of disease, and upon removing the wax from one, it was seen that a normal callus had formed over the wound.

*The parasite may penetrate uninjured melon vines.* On August 2 a few inoculations were made in the greenhouse to test the penetration powers of the parasite. A healthy vine was selected and bits of mycelium from the formerly used set of pure cultures were placed on the uninjured surface of the main axis; three in crotches and one on an internode. August 8 the fungus had evidently penetrated at two of the inoculated points, for the usual water-logged appearance on both sides of the covering of grafting wax was present. About two weeks later (August 22) the disease had progressed some distance above and below the place of infection at both of the above noted cases, but the one at the node seemed to have penetrated deeper than that at the internode. The nodal infection looked darker and had the usual quantity of resin-like gum on its surface, while the one at the internode left the vine looking a little light-grey, with scarcely a trace of gum. But both had fruit-

ing bodies of the fungus, although the darker one had them standing much closer together. This test shows plainly that the fungus is able to penetrate the healthy uninjured surface of the muskmelon vine, and it also seems probable, from this experiment, that natural infection, occurring only at the nodes, is simply due to the fact that the spores were held at those points by drops of water sufficiently long for them to germinate and penetrate the tissue of the host, while the internodes afforded no such halting places for water.

*Inoculation of melon vines in the garden.*—It seemed rather important that inoculations should be tried out in the open and at a place where the disease had not, so far, appeared. The Director of the Station, Dr. W. H. Jordan, kindly consented to sacrifice some muskmelon vines growing in his garden, and on August 2 three vines were inoculated with mycelium from the same set of cultures as that used in the greenhouse on the same date; but the fungus was put in scalpel wounds instead of on uninjured surfaces as was done in the greenhouse inoculations. Each vine was inoculated at two points and covered with grafting wax. Three check inoculations were prepared; one on each of the vines. They were re-examined August 8 and it was found that only two of the inoculations had been effective. Upon removal of the grafting-wax coverings from the unaffected inoculated points, a large part of the cortical region of the covered portion was found to have been destroyed. The fungous hyphae were found present throughout the dry portions of the cortex, but the deeper lying green portions of the vine contained no hyphae which could be seen in freehand sections.

*Microscopic characters of the pycnidial form.*—The fruiting bodies, upon microscopic examination, proved to be pycnidia containing two-celled hyaline spores of the Diplodina type. A section shows the pycnidia originating in the region just under the epidermis through which they break, finally seeming nearly superficial. The pycnidia are slightly depressed globular, with pore-like ostiola, and measure 100 -165 $\mu$. See Plate IV for

sketch. They originate from a very extensive, much branched brownish subepidermal mycelium and a much less profuse cortical mycelium. Both longitudinal and cross sections show the mycelium rather abundant in the cortical regions, but the deeper tissue reveals none except in cases where there is a wide gap in the sclerenchyma tissue, and even in such cases it does not progress very far between the bundles till some time after the maturity of the Diplodina spores. There were some cases noted in the greenhouse where the vines were killed about the time the spores were mature. Sections of such vines had abundant mycelium even in the pith region. A few days after such a vine had been killed by the disease a photograph was made of its cross section which clears up some points. See Plate IV.

*Discharge of pycnidiospores and how the disease was spread.* – Upon examination of the pycnidia with a hand lens the darker ones are all seen to have papillate elevations in place of pore-like ostiola. By the addition of a little water to some unbroken pycnidia under a cover glass the microscope showed the papillate elevations to be masses of two-celled hyaline spores which had been forced out from the interior of the pycnidia. Shortly, the masses loosened somewhat showing individual spores more clearly, streamed off into the water, with a rather compact column of like spores continuing the streaming as long as three or four minutes. If one now recalls that this fungous disease was preceded by an attack of red spider and then followed by a long daily siege of heavy spraying with hydrant water, it will probably help explain the rapid spread of the disease after it had once started. And it also seems probable that an explanation of the position, on the vine, of all infections may be sought here. During spraying the pycnidia were moistened and those having mature spores would send forth numerous streams of spore masses which were at once carried away by the continuous stream of water and lodged on other vines, even at some distance beyond, down which they would be washed by the descending water. As the axils of leaves and the crotches of branches would natu-

rally retain certain quantities of this descending water, loaded with spores, suitable conditions for their germination were thus secured, from which infection could take place by the penetration of the mycelium into the axils or crotches of the vines. Of course, the saturated and warm atmosphere of the house was also very favorable by permitting drops of water to remain even whole days at such places.

*Another spore form.* Around the nodal regions of some of the diseased vines are often darker fruiting bodies scattered among the brownish pycnidia, while a few regions were found having these black bodies alone. Only the older regions of diseased vines seemed to have the black fruiting bodies; for example, when any were present they were always most numerous about the point of infection, while farther removed from that point the pycnidia exclusively occupied the substratum (Plate III). The vines having any of these darker fruiting bodies were either dead or in a dying condition when first observed. These black bodies are perithecia, containing subsessils to sessile, aparaphysate, cylindrical to clavate-cylindrical, eight spored asci. They are very slightly imbedded in the host tissue as shown by hand sections, *i. e.*, they are evidently erumpent although finally nearly superficial. The perithecia are somewhat depressed-globular or inverted-top shaped with their papillate apexes perforated by ostiola. They measure about 100–165 $\mu$, and the asci 45–58 $\mu$.

*Collection and separation of the two types of fruiting bodies.* —A large quantity of material was collected, from the diseased portions of muskmelon vines in the greenhouse. Some specimens were dead and dry; others were from vines in a very much wilted and dying condition, while a few were taken from green, rather vigorous looking plants. It was only on the former two types of material that perithecia could be found. By going over all the collected material it was separated into two groups, one containing only pycnidia and the other from few to mostly perithecia.

LABORATORY CULTURES OF THE FUNGUS (IN 1907).

*Cell cultures of pycnidiospores.*—A number of culture cells were prepared with small drops of sterile water and a few (perhaps from three to twenty) pycnidiospores were introduced, which were secured by putting some crushed pycnidia into a test tube about one-third full of sterile water. Twenty-four hours after planting nearly all were germinated. Some camera drawings on Plate IV will help one get an idea of some of the stages as they occurred at this time. At the close of the second day there was some additional growth and branching, but the third day gave scarcely any growth although some differentiation had taken place. The short and sparsely branched mycelia had become septate and in many cases only a few of the terminal cells were filled with protoplasm. Most of the proximal cells seemed practically empty. Occasionally the two terminal cells on short lateral branches looked spore-like by being almost constricted off from the other cells of the mycelium. It has not been shown whether these bodies are secondary conidia or not; perhaps they are not. These cultures simply show that the pycnidiospores germinate in water and may be able to infect the host from twenty-four to thirty hours after a suitable environment has been attained. Transfers were made to potato-agar tubes to use for inoculations.

*Cell cultures of ascospores.*—Another set of fifteen culture cells were prepared like the above and the drops were each supplied with a crushed perithecium. Five infected drops were allowed to evaporate by holding the cover glass at some distance above a flame. A bit of agar was then put on the dried asci. All cultures were examined and those discarded which had no asci with spores. Both fusoid and constricted spores were often in the same drop, but in different asci. Two days after preparing the cultures many of the spores had germinated. There was practically no difference between the germination of these ascospores and the pycnidiospores. None of the fusoid spores were seen to germinate. The drops from three of the best cells were transferred to five potato-agar tubes. On the following day many of the germ tubes in the remaining culture cells

had become branched and septate. Only two of the agar cells had germinated spores. The others probably got too hot in drying over the flame. There was still no change in the fusoid spores. More transfers to potato-agar tubes were made.

FIELD INOCULATIONS (IN 1907).

*Ascosporic and pycnidiosporic inoculation of muskmelon.*—Two hills of muskmelons, which grew vigorous, healthy vines, having some full grown melon fruits attached, were selected in the garden, and on August 12 the plants of one hill were inoculated with pycnidiosporic and that of the other with ascosporic mycelium. The inoculating was done on the internodes by making tangential cuts into the cortex, inserting a bit of mycelium from potato-agar tubes and covering with grafting wax. Eight ascosporic and seven pycnidiosporic inoculations were made. Three checks were prepared for each group.

On August 24 seven of the ascosporic inoculations had produced the disease on the infected parts of the vine, showing the usual water logged conditions of the green tissue farthest removed from the center of infection. The cortex nearer the inoculations was dead and dry, having a greyish color, and specked with dry drops of gum here and there. The general appearance of all but one of the plants was fresh and green, in fact, aside from the dead cortical girdles at the points of inoculation, the vines seemed normal. However, one of the vines looked rather flaccid and its leaves drooped slightly. Only four of the seven points inoculated with pycnidiosporic mycelium had become infected, producing a result just like the one described above; but two of these infections had a few pycnidia at the edges of the grafting wax.

The plants were re-examined on September 16. The two formerly wilted plants and one inoculated with pycnidiosporic mycelium had died while two of those infected with ascosporic mycelium were very much wilted. All diseased parts contained fruiting bodies of the fungus. Both pycnidia and perithecia were present on several infections of each hill, but the asci

were, with few exceptions, without spores. Those having spores had the characteristic appearance, and were of the same size as those used for the cultures. The three checks for each group were normal and uninjured throughout.

*Inoculation of pumpkin vines with both mycelia.*—To get some idea regarding the possible parasitism of this fungus for other species of the gourd family, pumpkin (*Cucurbita Pepo*) cucumber (*Cucumis sativus*), and watermelon (*Citrullus vulgaris*) plants were inoculated with both types of mycelia.

On August 25 a vigorous pumpkin plant was inoculated in the usual way on various parts of its vine. Three inoculations with pycnidiosporic and four with ascosporic mycelium were made. September 5 two of the pycnidiosporic and three of the ascosporic infections had rather narrow bands of slightly water-logged tissue on both sides of the wax covering, but no traces of gum flow could be seen. September 14 they were again examined. The wax was removed from those having shown no trace of the action of the parasite.

The wounds made during inoculation had healed normally. The narrow bands of water-logged tissue on those mentioned above had become no wider but had become dry. A few of the covers were removed and a dead girdle of cortical tissue was found extending about the width of the wax. The more or less hollow pith region of one of these had the inner surface of its tissues of a brownish color. Some transfers were made of the pith tissue from two other specimens, which had had waterlogged areas about the wax, and a fungous growth resulted which seemed indistinguishable from the one employed in the inoculations. On September 18 there were scattered perithecia on the three remaining infections around which some dead tissue could be seen. The hollow pith region (around the edges of which was some brown tissue containing hyphae of the fungus) as well as the perithecia, may be seen on Plate VI. Upon consulting the labels attached to the above inoculations two were found to have been infected with ascosporic and one with pycnidiosporic mycelium, on August 25. The perithecia were of the usual type, size and color. There were no pycnidia

present on any of the specimens. Many of the asci had their spores nonseptate; others, however, were septate.

*Some peculiarities of pumpkin as a host.* Judging from the very slight injury done to the pumpkin vines there seems no doubt but that this host would be immune under ordinary conditions, though the presence of living mycelium in the naturally hollow pith region would probably have caused more trouble in course of time.

Perhaps the most striking peculiarity noted on this host is the entire absence of pycnidia from both ascosporic and pycnidiosporic infections. A large number of lighter-colored fruiting bodies farthest removed from the inoculations were carefully examined in the hope of their being pycnidia, but without exception, they were young perithecia in various stages of development. The substratum or host seems to be the determining factor as regards the production of perithecia and pycnidia and it would be of great interest to find some way of reproducing these conditions in pure cultures.

*Inoculation of watermelon vines.* — On August 31 inoculations with both types of mycelia on watermelon vines (*Citrullus vulgaris*), growing in the garden, were made. The infecting was done in the usual way by wounding the internodes of the vine with a sterile scalpel. Two vines were inoculated at two points each with pycnidiosporic mycelium and two other plants with ascosporic mycelium; one at three points and the other at two. Two checks were prepared for the group of four and three checks for the group of five. On September 5 all inoculations of both groups had taken and one vine in each group had wilted. Pycnidia were present on vines of both groups. They were the size and character of the Diplodina before mentioned; the spores also agreed in both size, form and color. Eleven days later, September 16, some of the dead and dying vines were collected. Specimens from both types of inoculation contained perithecia and pycnidia. The checks were all without any traces of disease.

*Comparison of susceptibility of muskmelon and watermelon.* —The parasite did more active and destructive work on water-

melon than on muskmelon. The watermelon vines were less resistant and the pycnidia were mature a day or two sooner than on muskmelon. The watermelon vines were healthy and bearing many but small fruits; however, the vines were rather short and slender. Possibly the severity of the attack was in some measure due to the rather slight vines. It would perhaps be of interest to grow some watermelons in a greenhouse with the fungus present, for it seems probable that they would be naturally parasitized by this fungus.

*Inoculation of cucumber vines.* On August 31, both types of mycelia were used to inoculate some mature but still vigorous cucumber vines. Three pycnidiosporic and five ascosporic inoculations were made, also two checks for each group. The method was the usual one; tangential cuts were made with a sterile scalpel into the cortex of the vines, and bits of mycelium inserted and covered with grafting wax.

September 5 there was no change either on the infected or the check vines. September 14 some of the wax coverings were removed and found to have the cortex of the vines uninjured by the introduced fungus. A normal callus had formed over the wound. All inoculated bits of vines were examined microscopically and cultures were tried from the interior but neither method revealed a fungus.

*Why is the cucumber vine immune?* Since the cucumber and muskmelon are species of the same genus (*Cucumis*) it was to be expected that the cucumber would also be parasitized. As there were no younger cucumber plants accessible, their resistance could not be tested. It seems very strange that this species of Cucumis should be immune while a species of a related genus is very susceptible. The similarity of stem structure of *Cucumis Melo* and *Cucumis sativus* is evidence that the difference in the susceptibility of the two species is not due to their structural differences but rather to differences in the cell sap or cell constituents.[31]

[31] Parasites, of course, differ in their reactions to the same groups of host species, as shown by G. M. Reed's work, Infection experiments with the mildew on Cucurbits, *Erysiphe cichoracearum* DC. Trans. Wis. Acad. Sc., Arts, and Letters **15**: 527–47 (1907), Madison, Wis.

INOCULATION OF POTTED MUSKMELON PLANTS.

*Inoculation of young muskmelon plants by pouring spores, suspended in water, over them.* Potted muskmelon plants which had been planted for this experiment (the Emerald Gem variety), had now attained to a size sufficient for use in inoculations in a moist chamber. They were from six to ten inches long, having from four to seven foliage leaves developed. September 2 six of them were put in a large cubical moist chamber, in the greenhouse, having its sides and top of glass and its floor covered with a layer of Sphagnum moss. The plants, floor and sides of the moist chamber were thoroughly sprayed with hydrant water. A quantity of crushed pycnidia was put in each of six test tubes about half full of sterile water and one tube slowly emptied on the vine and leaves of each of the plants. Also two of the potted plants were placed under separate bell jars in the laboratory. A tube of sterile water containing a large quantity of pycnidiospores was poured over one and the other left for a check.

September 5 there were a number of leaves, in both the cubical moist chamber and under the bell jar, having brownish areas where the tissue was dead. Sections of the leaves were made and found to contain mycelium threading through all parts of the affected regions. Many of the flower buds and leaf axils had traces of the water-logged tissue noted as a symptom of this disease. On September 7 most of the leaves of the inoculated plant under the bell jar were wilted and drooping. It was photographed and many of the above facts may be seen by referring to Plate V. The check under the other bell jar remained healthy.

September 22 only four of the six plants which were placed in the large cubical moist chamber had been practically killed. The other two had a few dead regions on some of their leaves but had seemingly recovered. Two of the dead plants had a few scattered pycnidia on the vines but none on the leaves.

*Inoculation with pycnidiospores by wounding the host.*—September 6, four of the healthy potted plants were inoculated with

pycnidiospores by placing the spores in a wound and covering with grafting wax. The pycnidia for this were carefully picked with a sterile needle and crushed in small drops of sterile water on sterile slides. The drop containing the spores was then carefully drawn off into the wound. One check was prepared. September 18, three of the plants were very much wilted and the fourth had only a small region next the wax which was water-logged. The three dying plants had numerous pycnidia, but no perithecia, on the diseased parts. On September 26 the fourth inoculated plant had wilted and was dying. Pycnidia were also present. October 7 perithecia could be found on two of the four. The check was still in good condition. On September 25 the two remaining potted plants were inoculated with ascosporic mycelium which had been obtained from Van Tieghem cell cultures, September 18. October 7 both vines were wilting and dying, and there were pycnidia present near each point of inoculation.

*The relation of the two types of fructification.* The pure cultures from the ascospores and the pycnidiospores, used in the foregoing inoculations, give strong evidence that the Diplodina pycnidia are the fruiting bodies of the Mycosphaerella, coming later. There seems no question but that these two types of fruiting bodies are simply stages in the life cycle of the same fungus. With but one exception, inoculation with ascoporic mycelium produced the Diplodina pycnidia before any perithecia were produced. The exception has but little weight here because on that host (pumpkin) the pycnidiosporic inoculation produced only perithecia also.

### SPORES, IN CULTURE, NOT TYPICAL.

On November 12, transfers of both types of mycelia were made to tubes of alfalfa stems. The mycelial growth of both was similar and vigorous, turning darker with age. November 26, numerous typical Diplodina pycnidia were present in all the tubes. No difference could be detected between those from the ascosporic, and pycnidiosporic mycelia; they were somewhat undersized from both. The spores were also small,

mostly 9–12 $\mu$. Only about half of them were septate. The shorter ones were either continuous or doubtfully septate.

## RE-EXAMINATION OF THE MYCOSPHERELLA WILT OF MELONS IN 1908.

*Introduction.*—Both the red spider and the Mycosphaerella wilt reappeared on muskmelons in the same greenhouse in 1908. The soil used on the melon benches in 1907 was removed in September, exposed to the weather, but returned to the same benches in March, 1908. The house was thoroughly fumigated with hydrocyanic acid gas before the plants were introduced. They were benched about two months earlier than in 1907.

*The appearance of other parasitic fungi.* When the melons were about two-thirds grown a Coremium-like fungus was found attacking two vines a few inches from the ground and destroying the cortical tissues on a short region of the stem. The fungus produced large quantities of spores but failed to infect other plants. The two affected plants neither wilted nor evidenced harmful effects, yet each had a girdle of destroyed cortical tissues. A Sclerotinia, probably *Sclerotinia Libertiana* Fckl., proved a more virulent parasite. The parasitized vines had an oily-green, water-logged appearance, and occasionally a few drops of exuded, resin-colored gum. By the presence of fluffy, aerial mycelium, later forming sclerotia, and by the entire absence of any fruiting bodies on the affected stems, it is easily distinguished from Mycosphaerella wilt, though it may cause partial wilting of the vines. Comparatively little damage was done by this fungus.

*Occurrence of the red spider.* The red spider became evident shortly after the appearance of the Sclerotinia; it was sprayed with water as in the previous season and readily controlled, though some leaves turned yellow.

*Reappearance of the Mycosphaerella wilt.*—About the time the first melon fruits had attained full size, the Mycosphaerella wilt reappeared; being about a month earlier than in 1907. On June 23, three vines were found to have some of their lower

nodal regions affected. The hardened drops of gum about the dry, greyish nodes with densely scattered pycnidia, and the oily-green, water-logged appearance of the stems below and above the nodes, was a repetition of its occurrence in 1907. Again, on some diseased vines the older affected regions (the parts having fruits of the fungus) were of rather dark color and had larger quantities of exuded gum. Numerous Diplodina pycnidia were present on all the affected nodal regions, and, on a few of the oldest infections, Mycosphaerella perithecia. A week after the disease was first noted, more than a dozen vines had become infected at several nodes each.

*No Phyllosticta on the leaves.*—A thorough search was made for Chester's Phyllosticta on the leaves of the infected vines but it was not found, though, at many of the diseased nodes the leaves died about the time the water-logged condition first was observed. (Probably due to the red spider.)

*The disease more conspicuous than in 1907.*—On July 20, at least one-third of the vines were either badly wilted or dead. The disease was more rapid and destructive than in 1907, and possibly more conspicuous because the red spider had injured and killed fewer leaves before the fungus appeared. The fuller descriptions of 1907 are referred to for details regarding the fructifications and appearance of the disease.

*Re isolation of the fungus in 1908.*—In order to obtain fresh, vigorous cultures for inoculation experiments, numerous tissue transfers from the interior of diseased, water-logged portions of muskmelon vines were made to tubes of potato-agar. Carefully picked perithecia and pycnidia[8] were crushed in separate drops of sterile water, and under sterile conditions, transferred to separate sets of 25 Van Tieghem cells. In about twenty hours, probably 95 per ct. of the spores, in both the ascosporic and pycnidiosporic cells, had germinated. Many transfers to tubes of potato-agar were made from both types of germinated spores. The resulting mycelial growths seemed identical in all tubes. An inoculation test on muskmelon vines proved both alike parasitic and followed by like pycnidia and perithecia.

[8] It is a comparatively easy matter to distinguish between pycnidia and perithecia with a hand lens.

MYCELIAL INOCULATIONS OF SOME OTHER SPECIES OF CUCURBITS.

Several other cucurbits were specially grown (1908) to be used in pathogeneity tests. Young potato-agar cultures were used as sources of the fungous mycelium. The stems of the plants were wounded by tangential cuts and the mycelium was placed under the sliver. About half of the inoculations were covered with grafting wax and the others were left without protection. (Both methods were effective to about the same degree.) In most cases but one set of inoculations was made before bacterial contamination of the stock cultures became evident, as shown by the inability of the fungous mycelium to parasitize *Cucumis Melo*. The following is a brief summary of the results:

1. *Cucumis sativus* (Cucumber). Twelve inoculations were made as stated above. No visible effect was produced on the host and no fungous fruits were developed.

2. *Cucumis Anguria* (West Indian Gherkin). Ten inoculations were made. The wounds healed normally and no fungous fruits resulted.

3. *Cucurbita maxima* (Boston Marrow Squash). Eight inoculations were made on the vines and six on the fruits. The wounds healed normally. However, some perithecia with spores and a few pycnidia appeared on the dead slivers of the vines, but the squash-fruits seemed neither affected, by such inoculations, nor developed any fungous fructifications.

4. *Cucurbita Pepo* (Pumpkin). There were eight inoculations made on the vines and seven on fruits, but no visible effects were produced on either, yet, a few of the dead slivers (resulting from the tangential cut preceding inoculation) on the vines developed typical perithecia with spores. No pycnidia could be found.

5. *Lagenaria vulgaris* (Sugar Trough Gourd). Forty inoculations were made on fruits and vines, with no apparent affect upon the host, but a few fully developed perithecia with spores were found on two dead vine-slivers.

6. *Luffa cylindrica* (Dish-cloth Gourd). Eight inoculations were made on the vines and five on fruits. No effect resulted on the vine but a few immature or sterile fruiting bodies occurred on a dead vine-sliver. Portions of two gourd fruits were decayed and contained mature Diplodina pycnidia with spores.

Though it is desirable to have these inoculations repeated, they are an indication that none of these cucurbits are normally susceptible.[32] The fungus had no evident effect on any but *Luffa cylindrica*, though it was able to vegetate and fruit on some dead vine-slivers of other species.

*Development of perithecia on the vines of naturally immune cucurbits.* It is unusual to find the natural sequence of spore forms interrupted or to have one entirely omitted. Spores of the perfect and imperfect forms of a fungus generally follow each other in some definite order, whether the perfect form be an oospore, zygospore, teleutospore or ascospore. But at present, there are some well known exceptions in each of the above groups: some producing but one known type of spore.

There has been some notable work done, on some members of the "oosporic group," in artificially causing either type of spore to be produced by means of certain culture media and environment,[33] and on species of the "zygosporic group," by showing them to be heterothalic and therefore requiring two sexually unlike individuals of a species for the production of zygospores.[34]

[32] Some recent infection experiments by Dr G. M. Reed give a comprehensive grouping of cucurbits and other plants in regard to their susceptibility to some parasitic fungi. Infection experiments with *Erysiphe cichoracearum* DC. Bul. 250,. Univ. of Wis. Science Series Vol. 3, No. 8, pp. 337–416), Madison, Wis.

[33] G. Klebs, Zur Physiologie der Fortpflanzung einiger Pilze, *Jahrb. f. Wiss. Bot.* **33**: 513 (1899), and also **35**: 80 (1900). G. Klebs, Willkürliche Entwickelungsänderungen bei Planzen, Jena 1903.

[34] A. F. Blakeslee, Zygospore formation a sexual process. *Science* N. S. **19**: 864–6 (1904) Lancaster, Pa. Sexual reproduction in the Mucorineæ. *Proc. Am. Acad.* **40**: 205–319 (1904), Boston, Mass. Zygospores and sexual strains in the common bread mould, *Rhizopus nigricans*. *Science* N. S. **24**: 118–122 (1906).

Whether the type of spores produced by the members of the felettosporic and ascosporic groups may be controlled by either or both of the above methods is yet an open question. In case of the Mycosphaerella wilt fungus, on its normal hosts, the environment seems the important factor. The cortical tissues have been thoroughly permeated by the mycelium and killed when the pycnidia are produced; and generally these tissues are comparatively dry before the perithecia appear. Some of the latter conditions are found on the dead pumpkin vine slivers, though others are absent. In this case it seems plausible to assume that conditions inhibiting further vegetative development of the fungus on diseased muskmelon vines, as well as the "adverse" environment of mycelium, introduced under a sliver of a pumpkin vine or similar host, tend to cause the production of the "perfect" spore form. The dry condition of the substratum *seems the* important factor in the formation of the "perfect" spore form. Lakon concludes that transpiration is the greatest single factor in causing the development of the fructification of *Coprinus plicatilis*, other factors being of importance only in so far as they accelerate transpiration. But in an *anhang* to his paper he states that his fungus would not fruit on *Vicia Faba* -decoction agar though it did when stems of *Vicia Faba* were placed in agar-agar.[85]

Some of the above observations on immune cucurbits and the pumpkin inoculations of 1907 (Plate VI) seem to indicate that certain conditions of environment may produce ascospores and be adverse to or check the formation of pycnidiospores.

INOCULATION OF MUSKMELON AND WATERMELON.

*Mycelial inoculations.*- About fifty inoculations were made in the field on each of both watermelon and muskmelon vines, which were large and vigorous. The mycelium, taken from a fresh potato-agar culture, was introduced under a vine sliver which had been made by means of a tangential cut with a sterile scalpel. Half of the infections were covered with graft-

[85] G. B. Lakon, Die Bedingungen der Fruchtkörperbildung bei Coprinus. *Ann. Myc.* **5**: 155–176 (1907), Berlin.

ing wax and the others left uncovered. They were all effective except four of the covered ones on muskmelon. Both pycnidia and perithecia occurred on these inoculations.

The stock cultures were retransferred to tubes of potato agar. A few days later another, but smaller, set of inoculations was made. Only a few of the uncovered ones were effective and produced pycnidia. The cultures were found to be contaminated with a bacillus, which after losing its motility developed great numbers of dumb bell-shaped and globular, brownish involution forms.

*Ascosporic and pycnidiosporic inoculations.* From freshly collected muskmelon vines (growing in the greenhouse) large quantities of pycnidiospores were obtained, suspended in sterilized water, and transferred to the plants to be inoculated, by means of a sterile pipette.

The vines from one muskmelon hill and one watermelon hill were inoculated. The uninjured nodes of each species were infected with spores. Every inoculated node was covered with wet, sterilized absorbent cotton. Equal numbers of similar inoculations were made on both hosts, except that the nodes were punctured with a sterile forceps before the spores were transferred to them.

The cotton on all nodes was resaturated with sterile water one and a half hours after inoculation. The following morning, fifteen hours after inoculation, sterile water was again applied. All but three of the cotton wads were still moist. Six hours later more water was added.

Only one of the forty inoculations became injurious and produced fruits of the fungus: on a punctured node of muskmelon.

Equal numbers of similar inoculations were made on other plants of the same hosts, using ascospores. The cotton was remoistened but once: three hours after inoculation. Neither the injured nor uninjured nodes were parasitized. The spore inoculations of 1907 (p. 218) no doubt were effective because they were put in direct contact with the inner host tissues and were provided with both moisture and nutritive matter under the protecting wax.

*A probable reason why the disease has not been found in the fields of western New York.* The almost complete failure of these spore inoculations, in the field, suggests a reason why the parasite has not been observed in the fields of this vicinity. It seems that the very moist, warm environment of the greenhouse is essential for the infection and development of this fungus and that field conditions are preventive.

REMARKS ON THE WORK OF 1907–1908; PREVENTIVE MEASURES.

As may be seen from this condensed summary of the work of 1908, excepting some additions and extensions, it is only a repetition of that recorded for 1907. In general, there is no difference between the two seasons' observations: the characters of the disease, as well as the two spore forms of the fungus, were the same throughout.

It seems probable that the spores of this fungus are not killed by exposing infested soil to weathering conditions of winter, nor by fumigation with hydrocyanic acid gas, and, that earlier planting of melons fails to prevent an attack. However, it seems that this disease may readily be prevented in the greenhouse by spraying thoroughly with bordeaux mixture when the plants are about half grown (*i. e.*, before the disease appears), and repeating often enough to keep them covered with the spray. An unsuccessful spraying experiment, on watermelons, is recorded by Mr. Chester,[36] but the failure very probably was due to the fact that the disease had become evident before the spray was applied.—When the plants are sprayed with water the house should have good ventilation.

## CONCLUSIONS REGARDING THE NAMES OF THE FUNGUS.

From Chester's characterization of the disease it appears possible that he also had the fungus discussed in this bulletin, on the same vines on which he found his Phyllosticta, though he mentions no fruits of it as occurring on the vines. Smith unquestionably had the Mycosphaerella-wilt fungus, but since

[36] Del. Exp. Sta. 5th Ann. Rpt., p. 79.

it has not been found on the leaves (the fruiting bodies therefore not occurring in spots), there is no reason to put the pycnidial form in Ascochyta. For these reasons it is retained in Diplodina. Smith having transferred the species name of Chester's Phyllosticta to both spore forms, it is used, though his description of the imperfect form must be discarded, and his characterization of the perithecial form is very general, scattered and incomplete.

## TECHNICAL DESCRIPTION OF THE MYCOSPHERELLA-WILT FUNGUS.

**Mycosphærella citrullina** (C. O. Sm.) Grossenbacher.

*Sphærella citrullina* C. O. Smith, Delaware Experiment Station Bulletin 70 (1905), Newark, Del.

*The perithecia* are roughish, dark brown to black, depressed-globular to inverted top-shaped, with, generally, papillate ostiola, simple, densely scattered, erumpent and finally almost superficial, 100–165 $\mu$. The asci are cylindrical to clavate-cylindrical, aparaphysate, sessile to subsessile, 45–58 x 7–10 $\mu$. Spores, mostly uniseriate, two-celled, hyaline, oblong-fusoid to fusoid, with one or both cells bluntly ovoid and therefore constricted at the septum (distal cell often larger) or not constricted.

On the vines of *Cucumis Melo* and probably *Citrullus vulgaris*.

**Diplodina citrullina** (C. O. Sm.) Grossenbacher.

*Ascochyta citrullina* C. O. Smith, Delaware Experiment Station Bulletin 70 (1905), Newark, Del.

*The pycnidia* are slightly depressed-globular, with pore-like ostiola, and of light brown to dark brown color; subepidermal to cortical erumpent and simple, 100–165 $\mu$. Spores, hyaline, two-celled, more or less cylindrical, with rounded or tapering ends. They may or may not be straight or constricted at the septa, 10–18 x 3–5 $\mu$.

On the vines of *Cucumis Melo* and probably *Citrullus vulgaris*.

# APPENDIX.

## THE IMPLICATED MYCOSPHÆRELLA (LÆSTADIA) CUCURBITACEARUM (SCHW.)? CKE.

The confusion wrought by Mr. Smith's last paragraph (p. 201 of this bulletin) brings out some things of interest. Though the fungus or fungi involved seem not to be the same as the parasite discussed in this bulletin, it is thought advisable to make an attempt at clearing up the difficulty. He has simply condensed the complications, to be found in Saccardo's Sylloge Fungorum and similar publications, regarding *Sphæria Cucurbitacearum* Fries and like named species found or described by others. The original specimens not being accessible, it becomes necessary to use only the published references of the names involved and therefore only suggestive conclusions may be given. Thanks are here expressed to Dr. E. J. Durand for suggestions regarding the reference "No. 1699 in Schw. Syn. N. Am. Fungi," to Prof. G. F. Atkinson for lending a specimen of Roumeguere's, labeled *Læstadia Cucurbitacearum* (Schw.) Sac., for examination, and also to the Director of the Royal Botanic Gardens, for a statement regarding specimens in the Kew Herbarium.

*Sphæria Cucurbitacearum* Fries published 1882, in Systema Mycologicum, II, p. 502, seems to be the original species; later de Schweinitz found what he took to be the same fungus and published it under that name as No. 1699 in his Synopsis of N. Am. Fungi (1831), referring it to Fries, though he fails to give the correct reference in Systema Mycologicum.

In an article,[37] appearing March 1883, "On Sphærella and its Allies," Dr. M. C. Cooke describes, among others, "*Sphærella* (*Læstadia*) *Cucurbitacearum* (Schwein. No. 1699); *Sphæria cucurbitacearum* Fr. Sys. Myc. II. 502." The article purports to contain additions and corrections to Volume I of

[37] *Journ. Bot.* 21:71, also copied in *Hedw.* 22: 137.

Saccardo's Sylloge Fungorum. This species is an addition; it seems to be an enlargement of Fries's description, with the addition of ascus and spore characters. In a note he says: "The sporidia are not mature, but the endochrome is divided, and there is every probability that they are uniseptate when mature; in fact, in some instances they appear to be so now; but this cannot be affirmed positively, although a figure beside the specimens in Herb. Berk. represent the sporidia as uniseptate."

It seems probable that the specimens used for this description came from or were collected by de Schweinitz and that they had asci and spores which Cooke describes. No doubt this is the point of departure or origin of the ascosporic species from Fries's Phoma-like fungus. Cooke evidently supposed his to be the same as that of Fries, though, of course, the latter makes no mention of asci and spores. However, it is possible that one is an imperfect form of the other; such an assumption may not be made till they have been definitely connected either from fresh material or from authentic specimens in herbaria. The former method seems possible though the latter is unlikely because most of those old type specimens seem unreliable. Ellis[38] gives a note on this species saying: "The specimen in Herb. Schw. is without fruit; immature or sterile." The Director of the Royal Gardens, Kew, writes "that *Sphærella Cucurbitaccarum* Schwein. No. 1699 has not been found in the Kew Herbarium. A copy of the label of the only Schweinitz specimen of this species is given below. The specimen was originally in Berkley's Herbarium and there is a drawing at the side showing the aseptate spores 'S (*subtectæ*) *Cucurbitaccarum* F. 452 Herb. Schwein.!'" A specimen of *Læstadia Cucurbitaccarum* (Schw.) Sacc. by Roumegeure was examined and found not only sterile, but without even an indication of fruiting bodies.

Thus two seemingly distinct things are called *Sphæria Cucurbitaccarum*, in later publications, without clearly intimating that such complications exist. Saccardo, on page XXXIII of

[38] North American Pyrenomycètes 264, Newfield, N. J.

the addenda to Vol. I, in his Sylloge Fungorum Vol. II (June 1883), gives Cooke's description in full under the name "*Læstadia Cucurbitaccarum* (Schw.) Sacc.; *Sphæria Cucurbitaccarum* Schw. in Fr. Syst. Myc. II., 502;" and, in Sylloge Fungorum Vol. IX (1891), page 580, the species is repeated as "*Læstadia Cucurbitaccarum* (Schw.) Sacc.; *Sphærella Cucucurbitaccarum* Schw., in Fr. Syst. Myc., II, 502," with no changes except in the synonym. Ellis, in Ellis and Everhart's N. Am. Pyr., page 264, practically translates Cooke's description except "Gregaria," but names it *Læstadia Cucurbitaccarum* (Schw.) and gives *Sphæria Cucurbitaccarum* Schw., *Sphærella Cucurbitaccarum* Cke., and *Læstadia Cucurbitaccarum* Sacc., as synonyms. Of course, it is possible that both Ellis and Saccardo gave their descriptions partially from herbarium material though that supposition may be incorrect as regards Ellis, judging from his note quoted on page 228 of this bulletin.

On the other hand, in Sylloge Fungorum Vol. III, page 148, Saccardo gives an exact transcription of *Sphæria Cucurbitaccarum* Fries (Syst. Myc., II, p. 502), with the addition of spore length, under *Phoma Cucurbitaccarum* (Fr.) Sacc. Allescher expresses the same view in Rabenhorst's Kryptogamen —Flora Vol. I, part 6, page 284.

Now, from this brief view of the complications it seems probable that *Læstadia Cucurbitaccarum* (Schw.) Sacc. and *Læstadia Cucurbitaccarum* (Schw.) Ellis and Ev.(?), are *Sphærella* (*Læstadia*) *Cucurbitaccarum* (Schwein. No. 1699) of Cooke, and that the confusion has arisen because neither Saccardo nor Ellis properly credited Cooke with the description he gave.